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Cooperative Agreement #DAMD17-95-2-5001 was implemented 15 October 1994 to provide funding support for Royal Thai Army investigators at the Armed Forces Research Institute of Medical Sciences (AFRIMS) engaged in research activities in collaboration with US Army investigators. The principal focus of research under the agreement is directed to activities to prepare for development and testing of vaccine(s) for the prevention of HIV infection and/or disease. During the reporting period, research activities were directed in 3 primary areas 1) continuing study of the natural history of HIV infection/disease in Thais to define and establish endpoints for projected vaccine efficacy testing; 2) cohort development studies attempting to define an appropriate population (s) for vaccine testing: and; 3) conduction of phase I/II vaccine studies to determine safety and immunogenicity of potential HIV vaccines in Thais. Other efforts under the Cooperative Agreement during the reporting period included 1) animal care and handling , including multiple small animal species and a primate colony, in support of other ongoing research activities at AFRIMS, exclusive of HIV research; and 2) site maintenance activities in support of research activities including glassware and utilities support.

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Pricha Singharaj 8 Nov 96

PI - Signature Date

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I. INTRODUCTION

A. General

The Armed Forces Research Institute of Medical Sciences (AFRIMS) conducts research into infectious diseases with both military and public health relevance to both the United States and Royal Thai Governments. Studies leading to the prevention of HIV infections are of primary importance to the Royal Thai Army. In addition, malaria, dengue, hepatitis, Japanese encephalitis, scrub typhus, and infectious diarrhea are all areas in which the RTA have major interest

B. Preparations for HIV Vaccine Efficacy Testing

Infection with the human immunodeficiency virus, type 1 (HIV-1), which causes the acquired immunodeficiency syndrome (AIDS), is pandemic. Current estimates indicate that at least 19 million people were infected as of the end of 1995, with a projected 100 million by the year 2000. 80% of infections exist in the developing world. The epidemic is currently exploding in South and Southeast Asia with about 4 million infections estimated at the end of 1995, most of which have occurred in the past 5 years.

Efforts to prevent infection with HIV-1 are currently limited to education and behavioral change, including the use of "safer" sex measures such as condoms and limitation of sexual activities to monogamous relationships with monogamous partners. These measures have so far proved to have limited effectiveness. Vaccines for the prevention of HIV-1 disease and transmission have been under development for several years with testing beginning in the United States in both seronegative and seropositive patients in 1989 and 1990.

In 1990, researchers in the Department of Defense (DOD), among others, recognized the emerging HIV-l epidemic in Thailand which had first become apparent in 1989 in intravenous drug users (IDU's). An agreement was made with the Royal Thai Army Medical Component (RTAMC) at the Armed Forces Research Institute of Medical Sciences (AFRIMS) to embark on a program of preparation for eventual field-efficacy evaluation of an appropriate vaccine candidate(s) for the prevention of HIV-1 disease and transmission.

Since 1991, The US Army Medical Component (USAMC) and the RTAMC at AFRIMS have conducted descriptive epidemiological studies of prevalent and incident infection with HIV-1 in Royal Thai Army conscripts, thereby contributing critical data to the high level characterization of the HIV-1 epidemic in Thailand. In January 1993, AFRIMS opened a Joint Clinical Research Center (JCRC) for the conduct of Phase 1/II (safety & immunogenicity) trials of vaccine candidates in Bangkok. Since June of 1993, the HIV-1 research collaboration at AFRIMS has embarked on a program of cohort development to identify and prepare a population for eventual participation in the efficacy evaluation of an appropriate HIV-1 vaccine candidate.

Many of the regions of the world where the HIV pandemic is worst coincide with areas of current or potential deployment of American forces. HIV-1 is a sexually transmitted disease (STD) and hence poses a threat to forces deployed to areas where HIV-1 is epidemic. This lethal threat has

been realized among United Nations (UN) forces deployed on "blue helmet" (peacekeeping) missions to countries such as Cambodia and Mozambique. Additionally, over 8,000 prevalent cases of HIV-1 infection within the US military are projected to cost over $1 billion for health care services within the DOD system by the end of this century. Hence there is a clear military relevance to the development of preventive measures for the prevention of HIV-1 disease and transmission, including, especially, an effective preventative vaccine.

C. Studies Using Animals

Most of the diseases studied at AFRIMS, including malaria, infectious diarrhea, dengue, hepatitis, scrub typhus and Japanese encephalitis, involve the use of animals as models of human disease. Data from animal models can be used to predict the outcome of similar events in humans. These data are reliable and can be applied to various types of research including vaccination, pathogenesis, toxicology and therapeutic agent studies. For example, one study is used to screen potential therapeutic agents for their activity against malaria. The animal model chosen for this is the mouse, one of the lowest animals on the phylogenetic scale that can be infected with malaria and then used to determine the effectiveness of new treatments. This is important not only to the military, but also to the more than 300 million people worldwide who become infected with malaria each year. In some areas, the malaria parasite is resistant to all known treatments.

However, many studies must be performed in a higher animal species. Before vaccines or drugs can be used in humans, the FDA requires that they be tested in a non-human primate model. AFRIMS is the best resource in the Department of Defense to perform this type of testing. We currently have protocols approved to test two new malaria vaccines, a new Hepatitis E vaccine and a new dengue vaccine. These vaccines involve cutting-edge techniques in molecular biology for both vaccine development and vaccine delivery. The availability of modern investigative techniques coupled with the extensive animal model availability makes AFRIMS a unique facility to develop and explore the effectiveness of these new therapeutics.

D. Laboratory Science Support

The glassware section provides glassware cleaning and support to all science departments at AFRIMS. This support is of fundamental importance to the ongoing research activities at AFRIMS and includes stocking commonly used items of glassware and the proper disinfection, cleaning, and/or sterilizing of all laboratory glassware. The glassware section is also responsible for the daily production of sufficient quantities of pure deionized water for use in laboratory assays which cannot otherwise be performed properly

E. **Space and Utilities Required**

Funding under the cooperative agreement is also directed by the Principal Investigator to the provision of site maintenance including space and utilities management for both the RTAMC and the USAMC in support of research activities.

II. BODY

A. General

Efforts made under the cooperative agreement during FY96 are focused in three general areas: 1) preparations for HIV vaccine efficacy testing; 2) animal care and handling in support of ongoing research at AFRIMS; and 3) site maintenance and laboratory support activities. In general, efforts have been successful, in spite of many scientific and non-scientific obstacles.

B. Preparations for HIV Vaccine Efficacy Testing

1. Natural History Study

a. Introduction

Understanding the natural history of HIV-1 infection is essential to planning for a phase III vaccine trial. There are many possible outcomes in the vaccinated subject who subsequently becomes exposed to HIV-l. In the best case scenario, HIV-l vaccines may prevent infection (sterilizing immunity) . However, protective vaccines (e.g. live attenuated polio vaccine) are thought to provide their clinical benefit through limiting (but not preventing) virus replication after challenge. Hence, although the induction of sterilizing immunity may be the ideal outcome in an HIV vaccine study, a product which induces an immune response which modifies viral replication, disease progression, or subsequent transmission is the more likely outcome.

Conceivably, vaccinees who are subsequently exposed to HIV-1 may demonstrate a booster effect of the immune response without infection, transient abortive infection, low grade controlled infection with a low viral load, unchanged symptoms of infection and viral load or, in the worst case, infection with higher than expected viral load, more severe symptoms and accelerated disease.

Valuable information about the natural history of HIV infection has come from prospective follow-up of cohorts of people at high risk of infection: homosexual males, hemophiliacs and intravenous drug users. As those in these cohorts become infected with HIV-1, the progression of the disease can be followed prospectively from the start of the infection. Because the time since infection is such an important predictor of progression, it is vital to study an incident cohort, that is, a cohort of people whose date of infection is known.

Almost all incident cohorts being studied at this time consist of males in Europe or North America, in most cases of Caucasian ancestry and infected with HIV-1, subtype B. There are

many reasons to think that disease progression in the developing world might be different from that in the developed world, but there is very little data available to assess the question. Data from a prospectively followed cohort of commercial sex workers in Kenya show much more rapid progression of disease than has been reported in other cohorts. Hypotheses about the reasons for this difference are easy to generate and difficult to prove without following other seroincident cohorts in the developing world. No information is currently available about the pathogenicity of subtype E, the predominant subtype in Thailand and whether the disease progression of those infected with E is significantly different from those infected with other subtypes, especially subtype B.

b. Study objectives

(1) To characterize viral, immune regulatory and clinical sequelae in recently HIV-1 infected Thai men, during the first three years post-infection. These data may form the basis of efficacy endpoints in future prophylactic vaccine trials in Thailand;

(2) To characterize (genetically and serologically) circulating HIV-l from recently infected Thai's. These data may form the basis for selection of vaccine strain prototypes for use in development of Thai-specific vaccine constructs; and

(3) To assess virus specific and immune regulatory correlates.

c. Study methods

Study population

This protocol contains plans for study of three groups of subjects: a prospective study of a seroincident cases, a cross-sectional study of prospective cases and an evaluation of uninfected persons. The first groups will be followed in order to document the natural history of infection during the first few years after infection. The second study is a cross-sectional look at prevalent HIV-l patients representing the full range of HIV disease in Thailand. The third group will provide data on normal values for the Thai population and serve as a control group for the other two populations studies.

Seroincident cases

Persons with incident HIV infections from cohort studies in Thailand are recruited for this study. If willing, they sign a consent form to take part in the study. At that time they donate 50 ml of blood. The subjects also receive a physical examination and a brief questionnaire requesting information about their risk behaviors and recent medical history. The seroincident subjects are asked to return every 6 months for three years.

Seroprevalent HIV-infected Thai's

HIV-infected subjects who enroll in this study are referred to the AFRIMS clinic from local physicians collaborating in the study.

Thai's without HIV-l infection

Uninfected Thai's in the study include Royal Thai Army recruits and personnel who work at AFRIMS.

Laboratory methods

At the time of enrollment and at follow-up visits, a complete cell count (CBC) and lymphocyte immunophenotyping is done on all subjects. PCR is also conducted on seroincident and seroprevalent cases. Cells, plasma and sera are archived from each subject for future testing. Other testing, described below, will be done on a selected basis:

CBC and lymphocyte phenotyping

CBC and differential are measured using the Coulter MaxM counter. Lymphocyte immunophenotyping is performed using dual fluorescent staining and analyzed on the Facscan using Simulset software at AFRIMS.

PCR subtyping

Primary PBMC derived DNA is used for PCR typing. HIV-1 subtypes are differentiated by nested PCR using primers in the gp 41 *env* region. Second round primers differentiated clades B and E, with the amplification of a 287 BP product.

d. Results

Study enrollment

Incident cases	98
Prevalent case	368
Seronegative case	108

The results presented (Tables 1-2) here include incident cases enrolled in the natural history study and results from subjects who are being followed under different protocols which allow follow-up of seroincident cases, but whose blood are being tested at AFRIMS. Table 1 shows the basic characteristics of all three groups of subjects. Table 2 shows more detailed information on the characteristics of the incident cases.

Table 1 - Study population demographics

	Incident cases* n=98		Uninfected n=108		Prevalent+ n= 368	
	n	(%)	n	(%)	n	(%)
Age						
<20	1	(2)	0	(0)	0	(0)
20-29	89	(91)	77	(70)	204	(55)
30-39	7	(7)	23	(21)	129	(35)
40-49	-	-	7	(6)	33	(9)
Unk	1	(1)	1	(1)	1	(0)
Sex						
Male	84	(86)	81	(75)	237	(64)
Female	14	(14)	27	(25)	131	(36)
Home Region						
Central	3	(4)	10	(10)	84	(23)
Northeast	7	(7)	42	(43)	35	(10)
North	70	(71)	11	(11)	48	(13)
Bangkok	12	(12)	32	(32)	178	(48)
South	1	(1)	-	-	3	(1)
Unk	5	(5)	3	(4)	20	(5)

*includes subjects tested, who were enrolled in other prospective studies
+does not include prevalent cases for whom CD4 counts have been provided as a "service" to the Phramongkutklao Hospital HIV clinic

Table 2 - Summary of incident cases (n=98)

	n	(%)
Subtype (n=73)		
E	55	(75)
B	1	(2)
UN	17	(23)
Estimated month of seroconversion		
Before July 1993	69	(70)
July 93-Dec 93	11	(11)
Jan 94-June94	7	(7)
July 94-Dec 94	1	(1)
Jan 95-June 95	1	(1)
July 95- July 96	9	(9)
Estimated time since seroconversion*		
<12 months	62	(63)
12-23 months	34	(35)
>24 months	2	(2)
Number of follow-up visits		
1	63	(64)
2	18	(18)
3	7	(7)
4	10	(10)

* time from seroconversion to first enrollment

CD4 counts and percents in incident HIV-1 infections and in uninfected Thai's

A small percentage of incident cases have returned for 2 visits, therefore, a cross-sectional determination of CD4 counts by time since estimated seroconversion has been done. This indicates that CD4 counts and percents are lower in both infected and uninfected persons compared to persons in the US, but that the percent decline during the first year appears to be similar. Differences in CD4 counts and percents were also found between men and women in both HIV-infected and uninfected Thai's. Preliminary RNA viral load testing has also been performed, which demonstrates similar levels to those reported from HIV-infected persons in the US.

Assay Development and evaluation

Proliferation

Lymphoproliferation assays to standard mitogens and common microbial antigens are being evaluated. Responsiveness to Candida and PPD has been found to be inversely correlated to CD4 cell count. Efforts to measure responsiveness to HIV-1 antigens have been hampered by a lack of purified antigens, although there is some suggestion of envelope specific responses in HIV-1 infected patients with CD4 counts>400/cu mm.

FACSCount

A comparison was carried out of CD4, CD8, and CD3 absolute counts between the Becton-Dickinson FACSCount^{TM} and FACScan^{TM}. These data indicate that the FACSCount^{TM} provides an accurate alternative to the FACScan^{TM} for CD4 counts (R=0.98). Similar high correlation's were found for CD8 and CD3. Both HIV infected subjects and controls have been used and the excellent correlation (see below) suggests that the FACSCount can replace FACScan for routine absolute CD4 counts.

Parameter	CD4		CD8		CD4/CD8	
	SCAN	COUNT	SCAN	COUNT	SCAN	COUNT
Number	308		222		222	
Correlation [r]	0.98		0.97		0.98	
Mean	482	504	810	793	0.59	0.66
Min. Value	2	1	99	82	0.001	0.003
Max. Value	1544	1516	2524	2727	2.53	2.38

Comparison of subtype determinations

We have tested 54 natural history cases by V3 and FM PCR, and found 100% concordance. A panel of Thai specimens is being used in a three-way validation project incorporating an HIV serotyping assay, a nested PCR assay which differentiates HIV subtype B from subtype E (FM primers) and heteroduplex mobility assay (HMA) for genotyping. Over 30 specimens have shown 100% concordance between HMA and FM primer typing.

Characterization of *in vitro* growth kinetics of HIV subtype E

The *in vitro* growth characteristics of primary isolates of HIV subtype E have not been extensively studied. A project is underway to assess the growth of HIV subtype E in PBMC, with further characterization in monocytes and lymphocytes derived from PBMC. Factors such as the method of isolation (standard cocultivation, plasma or directly from PBMC) in relation to subsequent passages of the virus will be monitored, as well as the phenotype of the virus and the source of cultured virus on further infection.

Plasma viral RNA quantitation

A plasma HIV RNA quantitative assay is being developed for use on incident cases of HIV infection. The assay uses a reverse transcriptase step employing a *pol* oligonucleotide, followed by amplification of the cDNA using PCR (*pol* primers), and product visualization using ethidium bromide. Dilutions of a reference plasmid of known copy number serve as the positive control and are used for construction of a standard curve. The detection method is currently insensitive, with a minimal detection limit of 10^5 copies/ml. Liquid hybridization using ^{32}P and detection with a phosphorimager are under consideration. It is expected that this in-house method will be replaced by a commercial assay if shown to meet local needs.

2. Cohort Studies

Cohort development for Phase III trials is ongoing. Cohort development includes planning recruitment and follow-up mechanisms and determination of follow-up rates, HIV-l incidence, behavior and STD rates in the population. Data collected from routine HIV-l surveillance being conducted in the RTA, as well as several HIV-l cohort studies, will provide information concerning cohorts which might be suitable for Phase III trials. Because the HIV epidemic in Thailand is dynamic and there are rapid changes occurring in the society, the process of identifying a suitable cohort has been challenging. Feasibility studies in two cohorts were begun in FY95 and continued through FY96.

Prevalence and incidence of HIV-l infections among recruits in the Royal Thai Army at Prachuab Khiri Khan

a. Introduction

Numerous studies have focused on the incidence and prevalence of HIV-l infection among Royal Thai Army conscripts (Tahan Gahn) . RTA conscript populations are socio-demographically homogeneous as relatively advantaged populations are excluded from conscription. Conscripts tend to be from non-municipal areas, engaged in agrarian occupations, possess a primary school education, and come from a Buddhist background. Those studies examining risk factors, interventions, or follow-up have focused on recruits in the Northern region where the epidemic has been most prominent.

Prachuap Khiri Khan is the southernmost province of the Central region. Fort Thanarat, the major RTA installation in the province has conscripts from geographically diverse backgrounds. Conscripts who arrive for service in May generally come from the Central or Southern provinces, while those who arrive in May are drawn from the Northeast. Fort Thanarat was chosen because it had a large recruit population, increasing prevalence, predominantly non deploying units (to simplify follow-up), and a single large hospital responsible for care. Its geographically diverse population also permitted exploration of regional differences in epidemiology and behavioral norms. The start date for this study was July 1995.

b. Study objectives

(1) Study the prevalence and incidence of HIV-l infection in recruits stationed at Fort Thanarat, Prachuap Khiri Khan province, Thailand.

(2) Study the attitudes, behavior and follow-up patterns in the recruits.

c. Methods

HIV-l testing is being done at baseline and every 6 months. At each bleed, a questionnaire is administered to evaluate behavior and knowledge. Two different educational and behavioral intervention programs are being implemented, using a non-randomized, quasi-experimental design. The incidence of HIV-l in the recruits, over all and in the two intervention groups, will be determined, along with changes in knowledge and behavior over time. At the end of the follow-up period, subjects will complete a questionnaire to assess attitudes towards participation in vaccine trials. As a service and incentive to the conscripts, Hepatitis B immunization is being offered, along with treatment of prevalent cases of syphilis. Routine follow-up and care is provided for HIV seropositive participants in this study. The HIV care and behavioral interventions will be adapted by the fort hospital and continued after the study is completed.

d. Results

Number of subjects enrolled: 3839

Number of subjects to be enrolled: 3839

Enrollment was considerably more complicated than originally expected. Although these conscripts are assigned to units located at Fort Thanarat, many recruits actually serve with small detachments of these units located at distances from the bases. This situation, along with the high percentage of persons leaving military service without permission, has yielded a follow-up rate of about 75%, which is comparable to other RTA conscript cohorts that have been studied.

Development of the behavioral interventions, particularly the small group activity-based intervention has progressed more slowly than expected. The complex organization of the fort and the large number of other research activities carried out by our staff made it difficult to beginning implementing the small group. Both intervention phases were begun during FY96, along with the Hepatitis B immunization and syphilis treatment.

Analysis of baseline data has begun, which resulted in a poster at the XI International Conference on AIDS in Vancouver. The poster reviewed factors related to HIV prevalence. It indicated that factors such as CSW contacts, sex with girlfriends, and injection drug use were related to HIV infection, with some variation across regions. Preliminary incidence data suggest that annual rates of HIV incidence at Fort Thanarat are probably below 1.0%. If this trend continues, then it is unlikely that this population will be considered for Phase III vaccine trials, although it will provide useful data regarding HIV epidemiology which may assist in any future cohort efforts with young adult males. Data collection at Fort Thanarat will continue through FY97 and will be completed during the first quarter of FY98.

Incidence of HIV-l infection among persons attending STD clinics and anonymous test sites

a. Introduction

This protocol studies the prevalence and incidence of HIV-1 infection in persons attending STD clinics in several areas of Thailand to determine whether this group would be a feasible cohort for HIV vaccine efficacy trials. The start date for the study was Sept 1995. The study is scheduled to be completed in April 1997.

b. Study objectives

(1) Study the prevalence and incidence of HIV-1 infection in persons attending STD clinics and anonymous test sites.

(2) Study the attitudes, behavior and follow-up patterns in the cohort.

c. Methods

Subjects are enrolled from STD clinics and anonymous test sites at three sites, Bangkok, Chonburi, and Lampang. Participants are tested for HIV-1 at 4 month intervals for one year. Education and counseling are provided at each visit. At each bleed, a questionnaire is administered to evaluate behavior and knowledge. At the end of the follow-up period, subjects will complete a questionnaire to assess attitudes towards participation in vaccine trials.

d. Results

Between September 1995 and February 1996, 1901 eligible persons were asked to participate in the study. Thirty percent of eligible men (371/1238) and 24% of women (161/663) agreed and were enrolled into the study. Among the 532 person who enrolled in the study, the HIV-1 seroprevalence was 3.4%. History of an ulcerative STD and lifetime CSW partners were associated with HIV-1 infection among men. There were no statistically significant risk factors identified for women. Follow-up at the second and third study visits has been 70-80%. To date, 3 incident HIV infections have occurred in study population.

3. HIV-1 Vaccine Testing

Screening and evaluation of potential volunteers

a. Introduction

Recruitment and screening of volunteers for HIV vaccine trials is necessary for the success of vaccine trials; however, the techniques and methods for successful recruitment for HIV vaccine trials were unproved and virtually untried in Thailand. Volunteers for all vaccine trials will be required to have clinical and laboratory characteristics which will be generally constant for all trials. Therefore, screening for potential vaccine trial subjects can be independent of the particulars anticipated vaccine trials. The ability to begin screening volunteers under a human use approved protocol, according to criteria which satisfy inclusion and exclusion criteria for the actual vaccine trial 30 to 50 days in advance of actual trial approval allows for a more rapid implementation and enrollment phase for each vaccine trial.

Information from this protocol is being used to guide future recruitment strategies. Additionally, information on normal lab values obtained in screening for the RV99 protocol has been useful in the design inclusion and exclusion criteria for future HIV research protocols in Thailand.

The protocol has recently been amended to include two new collaborating sites: the Vaccine Trial Centre at Mahidol University and Siriraj Hospital. Amendments are under review.

b. Methods

Recruitment and screening continued through December 1995. The next round of screening (for the upcoming Thai E vaccine protocol) is scheduled to begin Feb/Mar 97.

Evaluation of volunteers includes collection of demographic information, medical history, laboratory evaluation (including CBC, serum ALT and creatinine, HBsAg, pregnancy test, and RPR), chest x-ray, and in depth psychological and HIV-risk assessment.

c. Results

Bangkok: Over 200 telephone and personal inquiries resulted from the recruitment efforts. A total of 55 people were screened using a total of 58 screening numbers (three were reenrolled as the deadline for the validity of their lab results had run out). Of these, 27 were eligible and enrolled in the vaccine protocol RV99.

Chiang Mai: Over 400 potential volunteers have been contacted as a result of recruiting efforts. A total of 49 people were screened to enroll 27 volunteers in RV99.

Table 3 - Characteristics of volunteers in vaccine screening protocol

	BANGKOK (n=55)	CHIANG MAI (n=49)
mean age:	30 years (median=29)	32 years (median=32)
male:female	39:16 (71% male)	30:19 (61% male)
marital	35 single (66%)	20 single (41%)
status	12 married (22%)	22~married (45%)
	6 divorced (11%)	3 divorced (6%)
	2 widowed (4%)	4 widowed (8%)
education:	20 $\leq$ Senior Secondary (36%)	32 $\leq$ Senior Secondary (65%)
	25 $\geq$ University (64%)	17 $\geq$ University (35%)

A total of 54 people were subsequently enrolled in the RV99 vaccine trial, 27 in Bangkok and 27 in Chiang Mai. The most common reasons for exclusion were HIV seropositivity, lab abnormalities (high ALT and anemia) and volunteer withdrawal.

Phase I trial of Biocine HIV SF2 gpl2O/MF59 vaccine

a. Introduction

This double-blind, randomized, Phase I study evaluates the safety/tolerability and immunogenicity of the BIOCINE Human Immunodeficiency Virus (HIV) SF2 gpl20/MF59 Vaccine at the dose of 5Oug in two immunization schedules.

b. Study objectives

(1) Are there any acute adverse clinical reactions or laboratory evidence of toxicity to the candidate vaccine (i.e., local or systemic reactions or immunologic impairment)?

(2) Does the vaccine induce anti-HIV- I antibodies? In particular, does it stimulate anti-HIV neutralizing antibody to, at least, the homologous viral subtype (clade) represented in this product when administered to healthy volunteers?

(3) Is the safety, tolerability and immunogenicity profile comparable with two different immunization schedules?

(4) Does the candidate vaccine stimulate the production of a lymphoproliferative response to HIV- I as represented by antigens present in this vaccine candidate and other HIV- I envelope antigens?

(5) Is the safety and immunogenicity profile of this vaccine similar in Thai and U.S. citizens?

c. Study population

The study population consists of fifty-two (52), HIV-1 seronegative, healthy Thai adults enrolled from the community at two sites, twenty-six at (AFRIMS), Bangkok, and twenty-six at Chiang Mai. Each site had one drop out who was replaced, so a total of 54 volunteers were enrolled.

d. Methods

After receiving ethical and scientific approval from all relevant institutional review boards (IRB's) and from the ethics committee of the Royal Thai Ministry of Public Health and the Scientific Subcommittee of the Thai National AIDS Prevention and Control Committee, the trial was initiated on 29 August 1995.

Subjects were enrolled after being screened in the protocol established for screening and evaluation of potential volunteers (see section B.3 above)

Subjects were randomized to receive vaccine (n=20 each Group Al and B1) or placebo (n=6 in each of Group A2 and B2) . Two immunization schedules were compared. Volunteers enrolled in Group Al and A2 are vaccinated at 0, 1, and 4 months; volunteers enrolled in Groups B1 and B2 are vaccinated at 0, 1, and 6 months. Subjects were followed for four months after the third immunization. Subjects are asked to return for HIV testing every six months for three visits after the last study visit. In addition, all subjects will be contacted annually for a period of up to five years to monitor their general health after the last immunization according to guidelines outlined in the National Plan for AIDS Vaccine Development and Evaluation.

Subjects were observed for 30 minutes following immunization for evidence of immediate local and systemic reactions. They were instructed to watch for local (i.e., pain, fatigue, headache, nausea, myalgia, arthralgia, etc.) for seven days post-immunization. They were contacted by the study nurses within 24 to 72 hours post-immunization by telephone or by home visit to assess any symptoms reported. All adverse events were monitored until resolution. Vaccine immunogenicity was assessed by gp120 Enzyme-Linked Immunosorbent Assay (ELISA) and HIV neutralizing antibody assays, as well by specific lymphocyte proliferation. Immunogenicity of the two injection schedules was compared.

e. Results

<u>Study enrollment</u>
In all, 27 subjects were enrolled from AFRIMS (21 male; 6 female) and 27 from RIHES (17 male; 10 female). The mean age of subjects was 30 years in Bangkok and 34 years in Chiang Mai. There were two dropouts early after enrollment (one in Bangkok and one in Chiang Mai), both for personal reasons (BKK volunteer was incarcerated, CM volunteer found commuting difficult). There were no differences in frequency or severity of acute reactions between the placebo and vaccine groups.

Lymphoproliferation

The RV99 protocol requires antigen-specific lymphoproliferative responses of PBMC from the enrolled subjects. Due to logistic problems, a comparison was begun of lymphoproliferative responses of freshly processed versus cryopreserved PBMC from volunteers. PBMC from 21 subjects were processed- 13 at AFRIMS and 8 at RIHES. Antigen-specific lymphoproliferative responses to gp120 were clearly observed in both AFRIMS and RIHES study participants (total 11/21 - note that some of these subjects may have received placebo). Lymphoproliferation assays for RV99 were completed in Oct 1996 on 156 specimens.

gp120 EIA

The gp120 EIA assay has been successfully transferred to AFRIMS. Sera collected from all 52 subjects prior to vaccination and out to 4 months post third gp120/ placebo vaccination (312 samples) have been tested.

Reactogenicity

There were no serious or unexpected adverse events any of the volunteers.

4. Surveillance

a. Introduction

A previous nationwide seroprevalence survey with demographic data collection was conducted on Royal Thai Army conscripts from November 1991 to May 1993. This survey allowed definition of the epidemic nationwide and has assisted both the Ministry of Defense, the Ministry of Public Health, and other Royal Thai Government agencies to better understand the epidemic in Thailand.

This project studies the prevalence nationwide among recruits serving with the Royal Thai Army in Thailand and will assess temporal, geographic and demographic correlates of HIV-l infection among the young men. The information obtained from this study will help monitor the epidemic and assist in identification of location for potential cohorts for Phase III trials.

b. Methods

Demographic information is collected on young men entering service with the Royal Thai Army (RTA) nationwide and is merged with routine serologic HIV data collected by the RTA. The recruits are bled at entry into the RTA (every November and May) . Sera are testing for HIV by ELISA and positives are confirmed by Western Blot.

In 1996, serotyping of all HIV positive sera was initiated using a V3 peptide ELISA. In addition, a comparison of serotypes in a random sample of recruits from each regions in 1992 and 1995 was performed.

Data from this study will be analyzed, along with data from RV70 (a previous project which had a similar design) to evaluate trends in nationwide seroprevalence.

c. Results

Enrollment: 90,000 recruits

Subjects to be enrolled: 180,000-210,000

Trends in seroprevalence in the RTA (See Table 4)

Compared with 1993, the prevalence of HIV-1 infection in RTA recruits has decreased nationwide. The decrease was observed in all regions and at all educational levels. These data have provided the best indication of the success of the national HIV control program in Thailand.

Serotyping

Over 90% of prevalent infections in 1992 and 1995 were subtype E.

Table 4. HIV-1 seroprevalence and percent change by demographic factors for May 1993 and May 1995

	May 1993 Positive/Total (%)	OR (95% CI)	May 1995 Positive/Total (%)	OR (95% CI)	Percent Change
Number of recruits tested	1115/27913 (4.0)		726/28406 (2.6)		-36
Age (years)					
21	970/25221 (3.8)	1.0	605/25177 (2.4)	1.0	-38
22-29	103/2028 (5.1)	1.34 (1.08,1.66)	99/2200 (4.5)	1.91 (1.53,1.39)	-11
Level of education (years)					
0-6	758/18333 (4.1)	1.0	474/16598 (2.9)	1.0	-31
7-9	243/6340 (3.8)	0.92 (0.80,1.07)	185/7109 (2.6)	0.91 (0.76,1.08)	-32
10-12	64/2320 (2.7)	0.66 (0.50, 0.86)	42/3244 (1.3)	0.45 (0.32,0.62)	-53
13-16	5/2123 (2.4)	0.56 (0.20,1.41)	3/379 (0.8)	0.27 (0.07,0.87)	-66
Marital Status					
single	852/21082 (4.0)	1.0	530/21328 (2.5)	1.0	-39
married	221/6212 (3.6)	0.88 (0.75,1.02)	174/6049 (2.9)	1.16 (0.97,1.36)	-19
Area of residence in previous 2 years					
Rural	421/12029 (3.5)	1.0	220/10286 (2.1)	1.0	-39
Municipal	514/11585 (4.4)	1.28 (1.12,1.46)	378/13008 (2.9)	1.37 (1.15,1.63)	-35
Region of residence in previous 2 years					
Northeast	98/4425 (2.2)	1.0	45/4057 (1.1)	1.0	-50
Bangkok	150/3994 (3.8)	1.72 (1.32,2.25)	122/4369 (2.7)	2.56 (1.79,3.67)	-26
Central	456/11885 (3.8)	1.76 (1.40,2.21)	308/10530 (2.6)	2.69 (1.94,3.73)	-24
South	60/2604 (2.3)	1.04 (0.74,1.46)	76/4016 (1.7)	1.72 (1.17,2.54)	-18
North	296/3952 (7.5)	3.57 (2.82,4.54)	136/3989 (3.2)	3.15 (2.21,4.49)	-54
Upper north	237/1946 (12.2)	6.12 (4.77, 7.86)	46/2331 (5.4)	5.12 (3.51,7.47)	-55
Lower north	59/2006 (2.9)	1.34 (0.95,1.88)	90/1658 (2.0)	1.79 (1.16,2.77)	-33
Region of service					
Northeast	139/5371 (2.6)		82/5831 (1.4)		-46
South	50/2734 (1.8)		51/2716 (1.9)		3
Bangkok	162/4060 (4.0)		129/4142 (3.1)		-22
Central	406/11064 (3.7)		315/11096 (2.8)		-23
North	358/4684 (7.6)		149/4621 (3.2)		-58
Upper north	224/1797 (12.5)		94/1769 (5.3)		-58
Lower north	134/2887 (4.6)		55/2852 (1.9)		-57

C. Studies Using Animals

a. Introduction

The Department of Veterinary Medicine provides support for multiple animal-based research efforts. To meet the needs of researchers, the Department breeds, maintains and employs a sufficient number of animals to support seventeen active animal-based protocols. At any given time, we house about 5,000 animals of 10 different species, including three non-human primate species and four rodent species.

b. Results

During FY96 care and handling were provided for 30,769 animals in support of 16 research studies as shown in table five.

Table 5 - Animal utilization, AFRIMS, FY96

Animal Species	Number of Animals	Number of studies*
Rhesus monkey	77	4
Mice	29,663	9
Hamster	732	2
R. rattus rat	208	3
Guinea Pig	85	2
Geese	4	1

*Some studies utilized multiple species, therefore the total in this column is > 17.

D. Laboratory Science Support

a. Introduction

The ready availability of proper cleaning and decontamination of laboratory glassware is a fundamental requirement for all science departments at AFRIMS. The glassware section currently supports 28 separate categories of glassware stock and stocks over 13,000 glassware items on a continuing basis. The glassware section is also responsible for the daily production of sufficient quantities of pure deionized water for use in laboratory assays. The glassware section also invariably complies with AFRIMS safety regulations to make sure that all hazardous waste materials be discarded in a proper place for environmental protection.

b. Results

During FY96, the glassware section received 50,235 items of glassware for cleaning and decontamination. They distributed 39,567 items for use by various departments at AFRIMS. All hazardous waste materials after decontamination either by chemical treatment or under sterilization process will be dumped in the proper place.

Table 6 - Average daily glassware use, AFRIMS, FY96

Department	Flasks	Bottles	Beakers	Cylinders	Tubes	Pipettes
Virology	60	140	60	20	500	150
Immunology	40	40	30	10	250	
Entomology	20	30	20	10		
Medicine	10	15	10	10		
Bacteriology	130	60	30	20	500	50
Retrovirology	10	10	20	5		

III. CONCLUSIONS

A. Preparations for HIV Vaccine Efficacy Testing

1. Natural History Study

Due to inherent design deficiencies and unfortunate timing, the natural history study has, to date, yielded few data, of relatively limited consequence or applicability and of limited use in understanding the true natural history of HIV disease in Thailand. The natural history study has been most useful as a tool for providing reagents for laboratory strengthening and development. It has also yielded potentially useful insights for further research. A revised natural history protocol is in preparation to better address the needs of long-term follow-up for describing the natural history of HIV disease and defining endpoints for vaccine efficacy testing and to provide a mechanism for adequately following and evaluating vaccine subjects who develop HIV infection during vaccine trials.

2. Cohort Studies

Two cohort feasibility projects have been implemented after 18 months of preparation. The intensity of effort and resources which such undertakings demand has only become apparent with experience. At the onset of serious cohort development efforts we had planned conducting feasibility studies among 3 or 4 cohorts. It has become apparent, in retrospect, that we are ill-equipped to initiate that number of cohorts in such a short period of time.

The single-most important ingredient in successful cohort projects is a solid base of support and trust within the collaborating institutions. The Royal Thai Ministry of Public Health (MOPH) and the network of ministry sponsored hospitals and clinics have been most cooperative at all levels of cohort development. Cohort development within MOPH facilities and with civilian subjects, have required considerable efforts to establish working relationships with key individuals, including the Deputy Minister of Health, the Director of the Division of AIDS and with numerous ministry officials at province, district and community levels. In the case of the RTA, it is with hospital and base commanders where we have needed to establish working relationships. This has been relatively simple, but time consuming and requires regular contacts to maintain our excellent working relationships.

Plans for FY97 for cohort development may include at least one additional feasibility study. Implementation of such an effort will depend upon inquiries being made at a number of locations in Bangkok and nearby provinces. Additional cohorts for possible feasibility studies will continue to be explored, recognizing the significant possibility that neither of the two (or three) cohorts currently under study may prove to be viable for vaccine testing.

3. Phase I/II Vaccine Trial

a. Subject Recruiting

Recruitment for this first trial was surprisingly easy, at both sites, especially given the relatively high degree of uncertainty which existed prior to the initiation of the study.

In Bangkok, most enrolled subjects heard about the trial from the radio or newspaper. The best response to advertising came from an article written by a locally prominent columnist in a popular daily newspaper. A smaller number responded to the distribution of posters and brochures. In Chiang Mai, presentations made by the Investigator and staff yielded the best response as did personal referrals by staff. In both locations there was a preponderance of blood donors who volunteered.

b. Laboratory Screening

The relative lack of data establishing reference normal values for basic clinical laboratory assays complicated the process of screening volunteers successfully. Specifically, normal values for immunophenotyping, serum chemistry and complete blood counts (CBC), including all measured parameters, are not well established. A significant amount of data has been collected from various protocols on normal lab values in Thai people. These new values were used in preparation of the protocol for the upcoming vaccine trial.

4. Surveillance

Active surveillance of RTA conscripts will continue. The data collected in this effort continues to provide one of the best windows to the dynamics of the HIV epidemic in Thailand. Future efforts are being planned to conduct serotyping, and possibly genotyping, of viral isolates to better define the virological dynamics of the epidemic, especially as regards the intrusion of new viral subtypes (e.g., subtype C) and shifting dynamics of the current subtypes, B and E.

B. Studies Using Animals

Animal-based research will continue to place a fundamental demand on Veterinary Medicine resources at AFRIMS. With expanding regulatory requirements; increasing sensitivity to animal-care issues; and a relatively constant level of ongoing or new animal-based studies, demands for a high level of animal care and handling will continue unabated and very likely increase in coming years.

C. Laboratory Science Support

The level of active research protocols, ongoing and projected will continue at historical levels or greater and will continue to require an active glassware section to meet the needs of highly technical and resource intensive scientific investigation.

IV. REFERENCES

A. Natural history study

(1) Prevention of HIV-1 infection in chimpanzees by gp12O V3 domain-specific monoclonal antibody. Nature 1992;355:728-30.

(2) Clerici M, Levin JM, Kessler HA, Harris A, Berzofsky JA, Landay AL, Shearer GM. HIV-specific T-helper activity in seronegative health care workers exposed to contaminated blood. JAMA 1994;27:42-46.

(3) Clerici M, Giorgi JV, Chou CC, Cudeman VK, Zack JA, Gupta P, Ho HN, Nishanian PG, Berzofsky JA, Shearer GM. Cell-mediated immune response to human immunodef iciency virus (HIV) type 1 in seronegative homosexual men with recent sexual exposure to HIV-1 J Infect Dis 1992;165:1012-1019.

(4) Goedert J J, Kessler CM, Aledort LM, Biggar RJ, Andes WA, White GC, Drummond JE, Vaidya K, Mann DL, Eyster ME, et al. A prospective study of human immunodeficiency virus Type 1 infection and the development of AIDS in subjects with hemophilia. N Engl J Med 1989; 321:1141-1148.

(5) Fahey JL, Taylor JM, Detels R, Hofmann R, Melmed K, Nishanian P and Giorgi JV. The Prognostic value of cellular and serological markers in infection with human immunodeficiency virus type 1. New Engl J Med 1990; 322:166-172.

(6) Munoz AD, Vlahov L, Solomon JB, Margolick JC, Bareta S, Cohn J, Astemborski and Nelson K. Prognostic indicators for development of AIDS among intravenous drug users. J. AIDS 1992; 5:694-700.

(7) Nagelkerke, N.J., F.A. Plummer, D. Holton, A.O. Anzala, F. Manji, E.N. Ngugi and S. Moses. Transition dynamics of HIV disease in a cohort of African prostitutes: a Markov Model approach. AIDS 1990; 4:743-747.

(8) Ward JW, Bush TJ, Perkins HA, et al. The natural history of transfusion-associated infection with human immunodeficiency virus: Factor influencing the rate of progression to disease. New Engl J Med 1989;321:947-952.

(9) Hofmann B, et al. Serum beta 2-microglobulin level increases in HIV infection; Relation to seroconversion, CD4 T-cell fall and prognosis. AIDS 1990;4:207-214.

(1O) Clark SJ, Saag MS, Decker WD, Campbell-Hill S, Roberson JL, Veldkamp PJ, Kappes JO, Hahn BH, Shaw GM. High titer of cytopathic virus in plasma of patients with symptomatic primary HIV-l infection. N Engl J Med.1991;324:954-960.

(11) Stein DS, Korvick JA, Vermund SH. CD4+ lymphocyte cell enumeration for prediction of clinical course of human immunodeficiency virus disease: A review. J Infect Dis 1992; 165:352-363.

(12) Lang W, et al. Patterns of T lymphocyte changes with human immunodeficiency virus Infection: From seroconversion to the development of AIDS. J AIDS 1989;2-63-69.

(13) Weniger BG, Limpakarnjanarat K, Ungchusak K, Thanprasertsuk S, Choopanya K, Vanichseni S, Uneklabh T, Thongcharoen P, Wasi C. The Epidemiology of HIV Infection and AIDS in Thailand. 1991;5(suppl) :571-585.

B. HIV Vaccine Testing

(1) Chiyanon Sesawet. Foreign Broadcast Information Service Report, 14 June 1994, p.9.

(2) Louwagie J, McCutchan F, van der Groen G, et al. Genetic comparison of HIV-l isolates from Africa, Europe, and North America. AIDS Res Hum Retroviruses 1992;8:1467

(3) McCutchan FE, Hegerich PA, Brennan TP, et al. Genetic variants of HIV-l in Thailand. AIDS Res Hum Retroviruses 1992; 8:1887.

(4) Mascola JR, Louwagie J, McCutchan FE, et al. Two antigenically distinct subtypes of HIV-l: viral genotype predicts neutralization serotype. J. Infect Dis 1994;169:48.

(5) Kahn J, Sinangil F, Baenziger J, Murcar N, et al. rgp 120 HIV-l vaccine clinical trial: Clinical and immunologic responses to human immunodeficiency virus-1_{SF2} gpl2O subunit vaccine combined with MF59 adjuvant with or without muramyl tripeptide dipalmitoyl phosphatidylethanolamine (MTP-PE) in non-HIV-infected human volunteers. AIDS Program at SFGH and the Univ of CA, SF; and The Biocine Company, Emeryville, CA.

(6) Blackwelder WC. "Proving the null hypothesis" in clinical trials. Controlled Clinical Trials 1982;3:345.

C. HIV Surveillance in Thailand

(1) Weniger BG, Limpakarnjanarat K, Ungchusak K, et al. The epidemiology of HIV infection and AIDS in Thailand. Aids 1991;5(Suppl 2) :S71-S85.

(2) Wangroongsarb Y, Weniger BG, Wasi C, et al. Prevalence of HTLV-III/LAV antibody in selected populations in Thailand. Southeast Asian J Trop Med Public Health 1985;16(4) :517-520.

(3) Ungchusak K, Thanprasertsuk S, Sriprapandh S, Chokevivat V, Pinichpongse S, Kunasol P. The national sentinel seroprevalence survey for HIV-l infection in Thailand. Thai AIDS J 1990;2:45-56.

(4) Ungchusak K, Sriprapandh S, Pinichpongse S, Kunasol P, Thanprasertsuk S. First national sentinel seroprevalence survey of HIV-l infection in Thailand. Thai AIDS J 1989;1:57-74.

(5) Anonymous. Update: December 1994. In: HIV/AIDS Situation in Thailand. Bangkok, Thailand: Division of AIDS, Department of Communicable Disease Control, Ministry of Public Health, 1995.

(6) Hanenberg KS, Rojanapithayakorn W, Kunasol P, Sokal DC. Impact of Thailand's HIV-control programme as indicated by the decline of sexually transmitted diseases. Lancet 1994;344(8917): 243-245.

(7) Nelson KB, Celentano DD, Suprasert S, et al. Risk factors for HIV infection among young adult men in northern Thailand. JAMA 1993;270(8) :955-960.

(8) Nopkesorn T, Mastro TD, Sangkharomya S, et al. HIV-1 infection in young men in northern Thailand. AIDS 1993;7(9) :1233-1239.